RELATIVE EFFECTIVENESS OF DEHYDRATION AND NEUROHYPOPHYSIAL EXTRACTS IN ENHANCING WATER ABSORPTION IN TOADS AND FROGS

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It is well known that the vertebrate neurohypohysis contains substances which can increase the permeability to water of the skin of amphibians and reduce the rate of urine flow. The same effects can be produced by dehydration or by injection of hypertonic solutions. It has, therefore, been suggested that these treatments act by stimulating the neurohypophysis to liberate so-called water balance hormone. Ultimately, neurohypophysial activity should thus be responsible for at least part of the renal and cutaneous effects of hydropenia in amphibians (Eliassen and Jørgensen, 1951; Ewer, 1952; Jørgensen, 1950; Sawyer and Sawyer, 1952). It thus seemed of interest to compare the relative effectiveness of dehydration and neurohypophysial extracts in enhancing water uptake. Experiments were made on the toad *Bufo bufo* (L.) and the frog *Rana temporaria* (L.).

Technique

Water uptake was measured as previously described (Jørgensen, 1949). The animals were placed in stainless steel wire cages and immersed in tap water. The rate of water absorption was determined by weighing animal plus cage at suitable intervals, generally one or one half hour. The animals were dehydrated in glass beakers covered by gauze, at a rate corresponding to a daily weight loss of about 10 per cent. Injections were given subcutaneously. Both mammalian and amphibian neurohypophysial extracts were used. Insipidin AB and Pitupartin AB served as mammalian preparations. Insipidin contains 20 international units of the pressor-antidiuretic principle per ml., Pitupartin 10 units of the oxytocic principle per ml. Insipidin has been found to be more effective than Pitupartin in enhancing water uptake in the toad. In the experiments on toads Insipidin was consequently used, 2–5 U being injected per 100 g. body weight to ensure maximum effect on water uptake (Jørgensen, 1950). In frogs the oxytocic principle is apparently more effective than the pressor-antidiuretic principle of commercial neurohypophysial preparations (Heller, 1945). In our experiments maximal rates of water uptake were induced by injection of about 5 U of Pitupartin per 100 g. frog. Extracts of the neurohypophyses of frogs and toads were made either in the usual way by extracting the minced glands for about 5 minutes with boiling 0.25 per cent acetic acid or by finely grinding the glands in a mortar with a little sand and a drop of Ringer solution. The comminuted tissue was suspended in Ringer solution and injected. Maximal stimulation of water uptake was obtained with amounts of ex-

tract corresponding to less than one-tenth of a gland from an animal of the same species and of the same size as that into which the extract was injected. In the present experiments extract from one-tenth of a toad gland was injected into each toad and one-fifth of a frog gland into each frog.

Results

The effect of hydropenia on the ability of toads and frogs to take up water through the skin varies with the temperature, the season of the year and other factors which have not been investigated systematically. An example of the large seasonal variations that can be found in the effect of dehydration on the rate of water absorption is given in Figure 1. Water uptake of normally hydrated toads

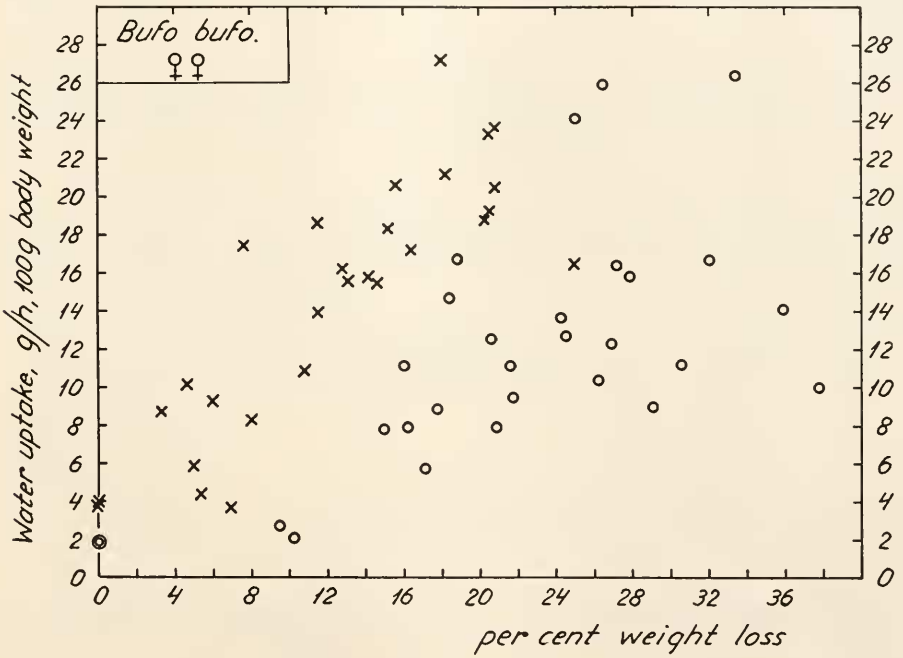

Figure 1. Water uptake in dehydrated female toads placed in tap water. × June; ⊙ December and March. Temp. 23–24° C.

was 4 ml./hr./100 g. in June and 2 ml. in December and March at 23–24° C. In June a weight loss of some few per cent strongly increased the water uptake, whereas in December or March a weight loss of 8–10 per cent was needed in order to affect the permeability to water. The maximal rates of water absorption, too, have been observed to be higher in summer-toads than in winter-toads. Comparisons between the effect of dehydration and neurohypophysial extracts should therefore be made at the same time of the year. Table I collates the results of a number of such comparisons between the relative effectiveness of strong dehydration and neurohypophysial extracts in enhancing water absorption in toads and frogs.

In experiments 2 and 3 of the Table the same animals were subjected to both dehydration and to injections. In experiment 1 the two types of treatment were given to separate groups of toads that had, however, been treated uniformly up to the time of the experiments. Desiccation corresponding to 20–30 per cent weight loss was much more effective than injection of supramaximal doses of neurohypophysial extracts in all experiments made at the same time of the year. There was no difference between the effect of mammalian and amphibian extracts. The pituitary extracts, however, produced larger effects during summer than during winter. In May–June toads responded as strongly to the extracts as they did to dehydration in November–December. No difference was found in the responses of the two sexes.

The increase in sensitivity to water loss and neurohypophysial extracts coincides with the onset of sexual activity in the spring. In toads imported from Italy in

TABLE I

Effect of dehydration and injection of neurohypophysial extracts on the rate of water uptake in toads and frogs. Water uptakes are expressed in ml. per hour and 100 g. body weight. The figures represent means of 5–15 independent determinations. Ranges are given in brackets

No. of experiment	Species	Time of year	Temp. °C.	Sex	Normally hydrated	Dehydration corresponding to 20–30% weight loss	After administration of:		
							Ringer's solution	Insipidin 2–5 U/100 g.	1/10 toad neuro-hypophysis
1	*Bufo bufo*	Nov. Dec.	17–19	♀	1.1 (0.8–1.7)	7.5 (5.1– 9.5)	1.3 (0.8–2.3)	2.0 (1.1– 3.3)	1.9 (1.1–2.9)
2	*Bufo bufo*	May	21–22	♀	1.7 (1.1–3.5)	18.7 (12.9–34.8)	2.0 (1.5–2.9)	8.1 (5.3–10.7)	
		June		♂	1.8 (0.9–3.6)	20.9 (9.0–38.8)	2.3 (1.4–2.8)	7.4 (4.4–11.3)	
								Pitupartin 5 U/100 g.	1/5 frog neuro-hypophysis
3	*Rana temporaria*	May	23–25	♀ ♂	2.4 (1.6–3.9)	19.6 (16.3–22.1)		4.8 (2.3–6.5)	5.3 (2.5–7.8)

February during the breeding period, 17–24 per cent weight loss resulted in water uptakes of 16–21 ml./hr./100 g. at 18–19° C. Extracts of 1/10 of a toad-neurohypophysis increased water absorption to the high values of 7–13 ml. At the same time Danish toads were still sexually inactive and showed the typical low "winter-response." The strong water balance reaction persists beyond the breeding period as may be seen from experiment 2 of the Table, performed one–two months after reproduction had taken place.

DISCUSSION

Dehydration was found always to be more effective than injection of neurohypophysial extracts in increasing the rate of water uptake through the skin of toads and frogs. If, therefore, enhanced water absorption after desiccation is produced by water balance hormone liberated from the neurohypophysis it follows that injected water balance principles must be less effective than hormone liberated

from the animal's own neurohypophysis. However, in man and dog Pitressin can produce a maximally concentrated urine and thus be as effective as naturally liberated antidiuretic hormone (O'Connor, 1950; West, Traeger and Kaplan, 1954). More likely, therefore, the effect of hydropenia on the permeability of the skin of toads and frogs is not exclusively due to increased neurohypophysial activity. At strong dehydrations the neurohypophysis is presumably of only minor importance in enhancing the ability to absorb water.

Summary

Comparisons have been made between the effect of strong dehydration (20–30 per cent weight loss) and of supramaximal doses of neurohypophysial extracts on the rate of water uptake through the skin of the toad *Bufo bufo* (L.) and the frog *Rana temporaria* (L.). Hydropenia increased the permeability to water much more than did injection of the pituitary extracts. Extracts of mammalian and amphibian glands were equally effective. The responses to desiccation and to injection of neurohypophysial extracts were larger during summer than during winter.

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